

AMENDED VERSION

IN THE CLAIMS:

1. (Currently amended) A method of assessing levels of oxidant stress by measuring ~~proteins—polymerized~~ non-aggregated by covalent ~~bonded~~ polymerized proteins formed after mixing oxidants with proteins and non-aggregated, covalent bond-polymerized proteins of biological specimens comprising the steps of:

separating non-aggregated proteins from aggregated proteins by difference in solubility of the proteins in buffer solution;

separating non-aggregated proteins by differences in the molecular mass using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or chromatography under denaturing condition of a purified and mixtures of proteins;

visualizing proteins with protein staining and Western blot analysis using antibody against the proteins, respectively; and

measuring increased levels of polymerized protein as an indication of increased levels of oxidative stress. ~~not including aggregates in vitro and in vivo~~
~~and indicating levels of oxidant stress based upon the measurements obtained.~~

2. (Original) The method according to claim 1, wherein said measuring step further comprises measuring nitrated, polymerized proteins.

3. (Previously amended) The method according to claim 2, wherein said measuring step includes measuring polymerized proteins selected from the group consisting of polymerized cytochrome c, nitrated-polymerized cytochrome c, 30 kDa cytochrome c, nitrated 30 kDa cytochrome c, 45 kDa cytochrome c, and nitrated 45 kDa cytochrome c.

4-9 (Cancelled).

10. (Currently amended) A kit for use in assessing oxidant stress ~~in-vitro and in-vivo~~ by measuring non-aggregated, covalent bond-polymerized proteins formed after mixing oxidants with proteins and non-aggregated, covalent bond-polymerized proteins of biological specimens comprising the steps of separating non-aggregated proteins from aggregated proteins by differences in solubility of the proteins in buffer solution;

separating non-aggregated proteins by differences in the molecular mass using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or chromatography under denaturing condition of a purified and mixtures of proteins; visualizing proteins with protein staining and Western blot analysis using antibody against the proteins; and ~~identifying a band on the gel and Western blot analyses with a molecular weight which when it produces a whole number when it is divided by the molecular weight of the monomeric form of the protein is an indication of oxidative stress in-vitro and in-vivo~~

measuring increased levels of polymerized protein as an indication of increased levels of oxidative stress.

11. (Original) The kit according to claim 10, wherein said assay further includes means for detecting nitrated polymerized proteins.

12. (Original) The kit according to claim 10, wherein said assay further includes means for detecting the formation of disulfide bonded polymerized proteins.

13. (Original) The kit according to claim 10, wherein said assay further includes means for detecting the formation of nitrated disulfide bonded polymerized proteins.

14. (Cancelled) A method of assessing oxidant stress by measuring nitritization of cytochrome c.

15. (Currently amended) A method of assessing levels of oxidant stress by measuring non-aggregated, disulfide polymerized proteins formed after mixing oxidants with proteins and non-aggregated, disulfide polymerized proteins of biological specimens comprising the steps of:

only measuring levels of a non-aggregated, disulfide polymerized proteins by separating non-aggregated proteins from aggregated proteins by differences in solubility of the proteins in buffer solution;

separating non-aggregated proteins by differences in the molecular mass using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDA-PAGE) or chromatography under denaturing condition of a purified and mixtures of proteins without breaking disulfide bond of polymers;

visualizing proteins with protein staining and Western blot analysis using antibody against the proteins, respectively, and measuring a level of a non-aggregated, disulfide polymerized proteins from the same sample by breaking disulfide bond of polymers by the addition of a chemical; and

measuring increased levels of disulfide bonded, polymerized protein by comparing results obtained with and without breaking bonds of the protein as an indication of increased levels of oxidative stress. ~~the formation of disulfide polymerized proteins not including protein aggregates in vitro and in vivo and indicating levels of oxidant stress based upon the measurements obtained.~~

16. (Original) The method according to claim 15, wherein said measuring step includes measuring the formation of nitrated-disulfide polymerized proteins.

17-35 (Cancelled).